Biennial Review Form

SOP Title: Tree Core Cutting Methods for Isotope Analysis

Originating Branch, or project, or group and number: WEB SIP EP.07 **Version**: 1.00
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BIENNIAL REVIEWS [a]

Date	EPA Reviewer (signature/title: PI, or project leader, or WACOR w/ WA#)
9/14/05	J. Renee Brooks

[a] **Signature documents the biennial review when no revisions are deemed necessary.**

If a modified version of this SOP is being followed these revisions should be submitted for review and approval.

A. Signature Page

Tree Core Cutting Methods for Isotope Analysis

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Version: 1.0
Serial Number:

Prepared by:

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B. Table of Contents

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C. Introduction

Stable isotope analyses of tree-rings can provide useful ecological information. However, this requires that the rings be separated. In some cases, separation may be required within a ring to examine seasonal differences. This experimental protocol (EP) is to document how tree rings are separated to avoid contamination between years.

Increment cores can be made in a variety of sizes and lengths depending on the need. Often a 5 mm core is sufficient for age dating and some sampling of tissue. However 12 mm increment cores are often desirable if tissue quantity is a limiting factor. Multiple cores per sampled tree will help in avoiding isolated occurrences and aid in cross-referencing.

Testing of wood core samples are separated into two different classifications. Core separation for non-mobile components (e.g., cellulose) and mobile components (e.g., nitrogen). All enriched cores are area restricted to greenhouse 8 or a low signature room in TERF.

D. Objective Statement

This experimental protocol (EP) was developed to provide standardized methods for the separation of wood core samples including sample labeling, core preparation, cutting technique and storage.

E. Equipment Needed and Location of Work

General Equipment

Scalpels
Rounded blades for scalpel (expect frequent changes of blades to facilitate good cutting)
Needle dissecting probe
Large forceps
Extra fine, polishing sandpaper
Baking tray with side lips
Cutting board, cardboard or poly cutting board
Plastic vials, 7 dram crystal w/ caps
Adhesive labels (Avery, Return address labels)
Magnifying crane arm lamp
Dissecting microscope
Plexiglas cutting shield for microscope platform
Fiber-lite for compound microscope
Tray style boxes for scintillation vials
Duct tape
Temporary or permanent location for disposal of cutting blades after each cutting session.

Use the duct tape to build up the handle in the finger grip area of the scalpel. This will help grip and provides some padding.

Specific non-mobile element equipment

Fine tip permanent markers (five distinct colors: black, green, red, yellow, and blue)
Soaking tray
Weight bar (wood cores will float other wise)
Paper towels

Work can be done in a lab or office environment as long as there is sufficient lighting and space. Normally a six by three foot table area is large enough to accommodate cutting work.

Specific mobile element equipment

Freezer paper
Metric ruler
Masking tape
Scissors
Squeeze bottle w/ 10% isopropyl alcohol
Kimwipes
Chemical spoon
Trash bags

F. Non Mobile Core Preparation

1. Once collected, the tree rings must be age dated according to International Tree Ring Database standards (http://www.ngdc.noaa.gov/paleo/treering.html). This work has generally been contracted to trained personnel. Difficulties with this step include missing, complacent, and false rings. Missing rings may lead to dating problems or lack of material to be tested, complacent rings change little from year to year, which makes cross dating with other trees difficult. Two growth periods can occur within a year, creating false rings. Dating of cores ensures that rings are accurately marked, particularly with long cores. If a core has been dated a common marking system is to use dots: One dot for a decade, two dots for a half-century, and three dots for a century. (Figure 1) An inferred missing ring will be indicated by two dots that are slightly offset, one on each side of the annual ring.

2. Review the notes from the person doing the cross-dating, concerning possible missing rings, wedge growth, or false rings or other anomalies.

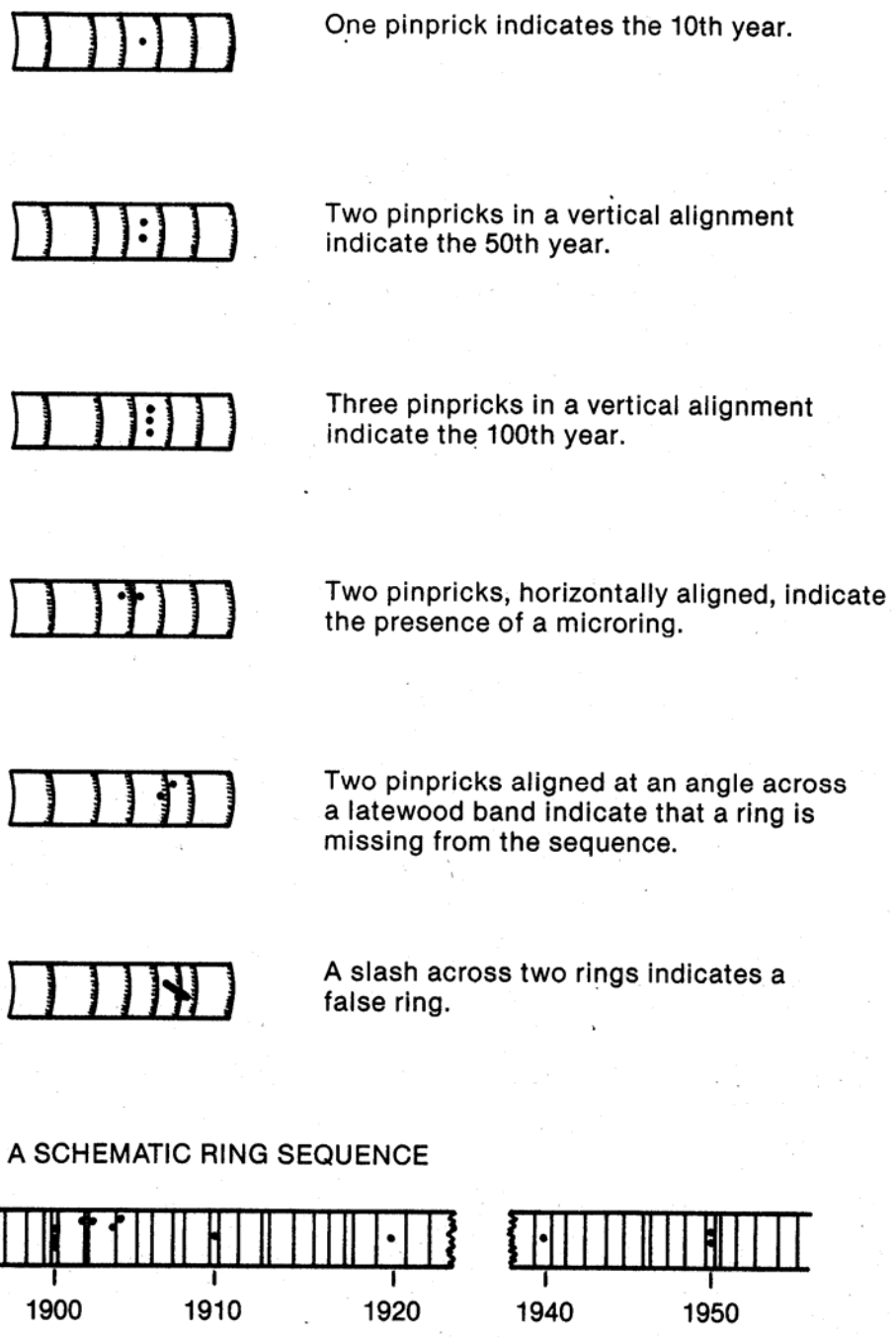

Figure 1: Method for noting 10-year, 50-year, 100-year rings and micro, missing and false rings. From figure 10 in Spruce Budworms Handbook, 1988.

3. Mark each annual ring with a color code. Five fine tip permanent markers are needed, green, yellow, red, blue, and black. Test to see that marks will not disappear when they are soaked. (Figure 2) Sanford ultra fine point "Sharpie" have worked well.

1999	* *	Green
1998	* *	Yellow
1997	* *	Red
1996	* *	Blue
1995	* *	Black
1994	*	Green
1993	*	Yellow
1992	*	Red
1991	*	Blue
1990	*	Black
1989	* *	Green
1988	* *	Yellow
1987	* *	Red
1986	* *	Blue
1985	* *	Black
1984	*	Green

Figure 2: denotes colors and marks for annual rings within a decade. These markings aid in keeping track of which ring is which in the process of cutting cores.

4. If the late and earlywood are to be separated then place the dot near the transition point from earlywood to latewood so that they will be cut in half later. Since each section is marked, if an error occurs by putting a piece into an incorrect vial there's a chance of correcting the error.

5. Photocopy the cores grouped together so that the last year of growth lines up. Orient the core flat sanded side to the copier glass plate. Put an identification label in the copier for each core or write it on the sheet after printing. The copy may come in handy later as a reference. The copies work well for discussing possible cutting problems because multiple people can see the section in question.

G. Cellulose Core Cutting Procedures

Work should be conducted on one tree at a time. If multiple cores are taken from a tree usually they are combined to increase sample size.

Preparing for cutting

1. Make vial labels: Each tree annual growth will be placed in it own vial. Vials should be labeled prior to cutting any cores with at least, site, identification number, year of growth and **E**arly or **L**atewood if they are being separated. (Figure 3) Each year will have two vials if annual growth is separated. Scintillation vial tray boxes work well for organizing samples.

   Plot5-327-99L Plot5-327-99E
   Plot5-327-98L Plot5-327-98E
   Plot5-327-97L Plot5-327-97E

   Figure 3: When annual rings are being separated into latewood (L) and earlywood (E), two vials will be used and labeled in such a fashion.

2. Examine annual rings for all cores from the same tree. Look for any unique features that may occur in only one of the cores and use the others for a reference. Also check the notes from the original dating analysis.

3. Sanding the cores can visually enhance the annual ring boundaries if needed. Use a very fine grain paper or wet/dry polishing sandpaper. Using a quality pencil or gum eraser after sanding will remove most of the sanding particles from the wood surface to give the best definition.

   - There is one real potential draw back with sanding. The sanding particles or the eraser might influence data analysis. The decision will have to be made for each study conducted. If sandpaper is used then a fresh piece should be used for each core to minimize cross contamination. Discuss sanding with PI before proceeding. This is a particular concern for enriched samples.

4. Wetting cores often shows more definition in the transition region of each annual ring. If the element being analyzed is non-mobile wetting can be used. Place the core or cores from a single tree in a tray of water deep enough to be submerged. Soak for an hour or two before starting to cut. Use some sort of weight to hold the cores down or they will warp. Soak for about an hour before starting work. Periods longer than three to four hours of soaking may cause the wood to swell to a point where definition is lost in growth transition and between annual growth. Rotate the cores to prevent over saturation.

When working with multiple cores from one tree do not cut one entire core then proceed to the next. Instead work them all back equally at the same time. Either cut one year at a time for each core or five years of annual rings for each core then move to the next.

Before starting a five year segment or single year compare all the cores and to make sure all aspects are similar. If a problem has occurred then only a small segment of data has been lost.

Cutting:

5. Remove a core from the soaking tray and dry excess water with paper towel.

6. Look at the annual rings with the aid of a magnification lamp or dissecting microscope.

7. For late and early wood separation: Each annual ring needs to be divided into two separate sections, early, (wood formed in spring) and late, (summer/fall wood). (Figure 4) Generally the coloration of earlywood is noticeably lighter than the darker latewood. Finding the boundary where one starts and the other ends can be difficult. At this point things become rather subjective but with practice a person can become very consistent.

   Hints for determining where to separate: The transition point where to cut is based on visually becoming familiar with a particular species, texture of wood, coloration change, resin characteristics and feel with the edge of a scalpel blade. The earlywood is made of long thin-walled tracheid cells. Under a dissecting microscope the earlywood looks kind of like a sawdust pile pressed together. Pieces easily fracture or flake off when the edge of a scalpel blade if drawn across the annual ring. Latewood is composed of small thick walled tracheid cells compressed tightly together that appear much darker in color. The blade of the scalpel will slide smoother across this surface than earlywood. The resin ducts can cause some confusion because of a darkening appearance in earlywood like the latewood. Look for the cellular texture of the wood, is it easily damaged with the blade edge. Based on the different characteristics found during the examination a cut must be made.

   Separating annual rings: If the blade of the knife is dragged gently across an annual ring to the end of the latewood and beginning of the next annual ring (earlywood) often a slight drop will be felt at the boundary point between the annual rings. Visually this transition from one year to the next is very defined as well. (Figure 4)

   Cutting under the microscope: When the annual rings are too fine to discern under the magnification lamp use a dissecting microscope. Protect the staging plate with a clear piece of plexiglass. At first cutting under microscope will feel awkward but with practice cutting gets much easier. The sharper the cutting blade the better. Cutting is a much slower process because of the precision needed. Apply less pressure than the normal cutting style and make several more passes around the core instead. When using a high intensity fiber light the outer annual ring may become slightly translucent depending on the angle of incidence, helping to discern the best cutting boundaries.

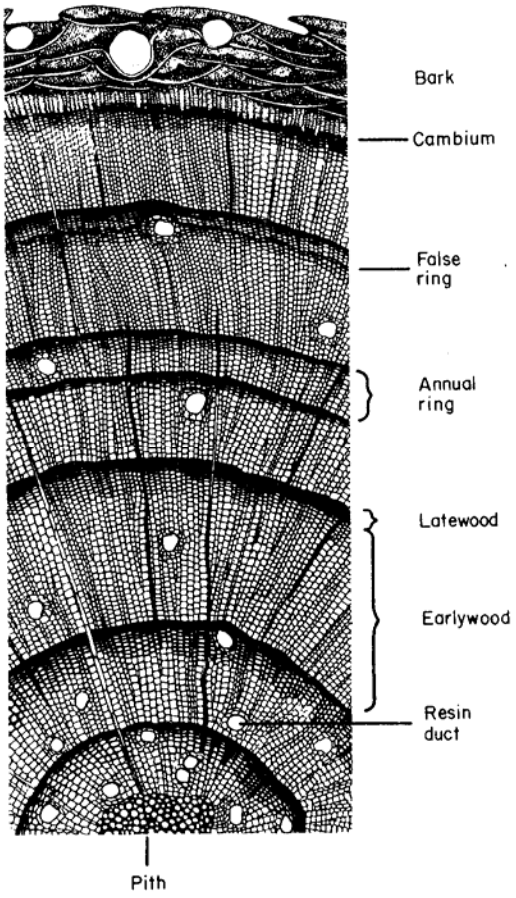

Figure 4: Drawing of cell structure along a cross section of young stem of conifer. Tracheids are the predominant cells in the xylem. The earlywood is made up of large relatively thin-walled tracheids, and the latewood is composed of small, thick walled tracheids. Variations in tracheid thickness produce false rings in either earlywood or latewood. The large circular openings are resin ducts, which form a complex system of tubes throughout a coniferous tree. From figure 2.3 in Fritts, 1976.

8. Orient the core so cutting will go from the most recent years of growth toward the pith.

9. Cutting a section will take several steps. First visually familiarize yourself with the section to get an idea of the path that will be needed to separate the sample.

10. Place the core on a piece of corrugated cardboard in a baking tray. The core is easier to handle on the cardboard and prevents the blade from hitting the metal baking tray and skittering. The cardboard will also absorb any excess water from soaking not caught by toweling off.

11. Hold the core in one hand and the scalpel in the other. Depending on the section of ring to be cut either work forward or backward around the core. The objective is to get the most precise cut possible. The forward cut works best when cutting between annual rings. Walk the blade tip forward with a rocking motion around the core to the starting point. The blade will flex enough to conform to most irregularities. The backward cut generally works best for cutting in the transition region between the latewood and earlywood. Roll the core forward and work the blade from the tip to back of the blade. Try to avoid lifting the blade from the core in order to get a continuous cut. The first cut is not meant to be extremely deep, it is only to establish the cutting path. Several more cuts around will be needed. A gentle prying motion after several times around may allow for a clean fracture sample piece.

12. Place the just cut early or latewood pieces into the appropriate vial. Do not put the cap on the vial at this time. They need to be dried before capping (see #18)

13. Clean the latewood face of the next annual ring so that no earlywood remains. Shavings from the cleaning are not to be kept but disposed of to prevent cross contamination.

14. At the end of a cutting session have all cores from a sample tree completed to the same point. Have a written log of the starting point and ending point for the cutting session along with any relevant information about any of the cuts. (Figure 5) Some annual rings will be too fine for even separating under the dissecting microscope. So the decision will have to be made depending on the study whether to place the entire annual ring in the earlywood or latewood vial. In this situation consult the project principle investigator (PI).

15. Remove the remaining sample core sections from the water soak and place on paper towel at the end of each day cutting.

16. Change the cutting blade at least once a cutting session.

17. Some cores may extend beyond the center point allowing for addition material to be used. If the decision is made to use this material make a note each time it occurs because sample weights will not represent a normal core set.

18. Place the sample vials in a drying oven for at least four hours then remove and put the caps on.

Project name: _____

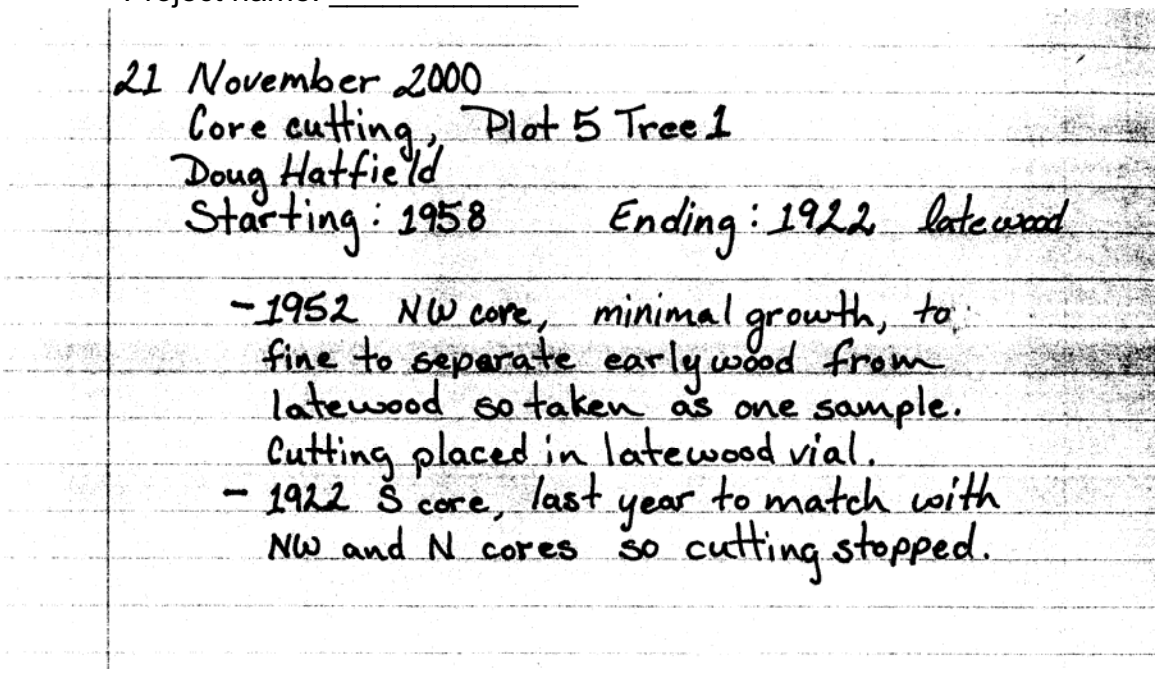

Figure 5: Example of how a normal journal entry might look for core cutting data for a specific project. Include: date, type of work done, who did the work, the starting and ending points, and any comments about the cores or cutting process for the day. Minor observations may prove to be very important later.

H. Mobile Isotope Core Preparation:

Introduction: The purpose of this procedure is to cut tree increment cores into segments representing major tissue types (outer bark, active phloem, sapwood and heartwood) so that uptake and translocation of mobile isotopes (e.g., ^{15}N) among tissues can be quantified.

1. Record all info on straw in a laboratory notebook designated as permanent record for this procedure for a specific project.

2. Label vials.

3. Open "straw" – take care not to break core, or mix bark, phloem, and wood segments making sure to keep pieces in order if already broken.

4. Measure segment lengths in (mm). Sand the core with #600 fine grit sandpaper if the annual ring boundaries can not be discerned by using the compound microscope. Remove excess dust from sanding but rubbing a clean gum eraser over the core. Be sure to remove any of the eraser particles.

- ♦ Segments to measure (See Figure 6): outer bark (B), active phloem (P), sapwood for the last five years of growth (S1 – 5), sapwood year six back from phloem to the heartwood marked with a permanent marking pen (S6 – H), and heartwood (H). Take two measurements on opposite sides of the core for the (S1-5) segment and average them. Use the same procedure to measure the (S6-H) segment Also take two total distance measurements of sapwood to heartwood and average them.

  - Active phloem is difficult to distinguish from bark after the core has dried. Therefore, it will be safest to collect as "phloem" only the first 5 mm of bark from the outer boundary of sapwood (i.e. cambium).

- ♦ For cores from the middle and upper sections of the trees, record total heartwood radius whenever the center ring or "pith" can be clearly identified.

5. Collect the (B) and (P) segments and place in their respective vials. Take care to remove soil and lichen from outer most bark if needed. Do this by sanding lightly with a 300 or 200 grit sandpaper to clean the surface.

6. Place phloem in vial.

7. Use a dissecting needle to mark (indent) earlywood of year 5 ring, then use a scalpel to slice off the (S1-5) segment, including latewood of year 5 and as much of the earlywood as possible. Take care to follow year 5 latewood all the way around core before cutting through.

8. Cut off the (S6-H) segment, but actually stop short of sharpie mark (may be contaminated with N-containing ink)

9. Measure 1 cm after sharpie pen mark on the heartwood then measure another 2 cm farther and cut the remaining length off past the 3 cm point. The 2 cm (H) segment is the one to keep.

10. Place heartwood sample in vial

11. Clean scalpel and tray before proceeding to next sample with 10% isopropyl alcohol.

12. At end of day transfer notebook data to an Excel file and drag copy to project folder on Nabu/Common server to back-up work. This Excel file will be used to collate the core segment data with the mass spec electronic data file following isotope analysis.

Wood core structure for cutting:

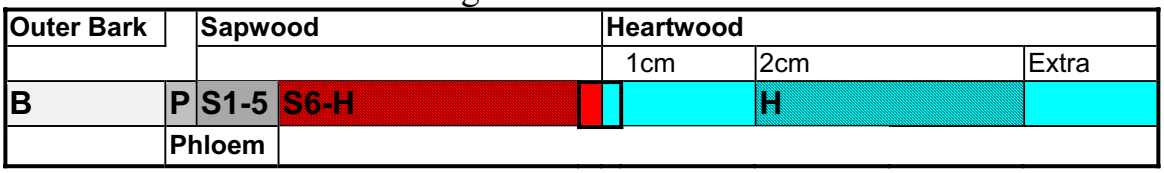

Figure 6: Structural sections of a typical core. Bark, phloem, sapwood 1-5 years, sapwood 6 years to heartwood, and heartwood are to be collected. Don't collect at the transition of sapwood to heartwood where marking pen has been used.

Bibliography

Spruce Budworms Handbook, "Using Dendrochronology To Measure Radial Growth of Defoliated Trees." United States Department of Agriculture, Agriculture Handbook No. 639, June 1985, August 1988.

Fritts, H. C. (1976). "Tree Rings and Climate." Academic Press Inc. New York, New York.